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Consumer-Phase *Salmonella enterica* serovar Enteritidis Risk Assessment for Egg-Containing Food Products

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We describe a one-dimensional probabilistic model of the role of domestic food handling behaviors on salmonellosis risk associated with the consumption of eggs and egg-containing foods. Six categories of egg-containing foods were defined based on the amount of egg contained in the food, whether eggs are pooled, and the degree of cooking practiced by consumers. We used bootstrap simulation to quantify uncertainty in risk estimates due to sampling error, and sensitivity analysis to identify key sources of variability and uncertainty in the model. Because of typical model characteristics such as nonlinearity, interaction between inputs, thresholds, and saturation points, Sobol's method, a novel sensitivity analysis approach, was used to identify key sources of variability. Based on the mean probability of illness, examples of foods from the food categories ranked from most to least risk of illness were: (1) home-made salad dressings/ice cream; (2) fried eggs/boiled eggs; (3) omelettes; and (4) baked foods/breads. For food categories that may include uncooked eggs (e.g., home-made salad dressings/ice cream), consumer handling conditions such as storage time and temperature after food preparation were the key sources of variability. In contrast, for food categories associated with undercooked eggs (e.g., fried/soft-boiled eggs), the initial level of *Salmonella* contamination and the $\log_{10}$ reduction due to cooking were the key sources of variability. Important sources of uncertainty varied with both the risk percentile and the food category under consideration. This work adds to previous risk assessments focused on egg production and storage practices, and provides a science-based approach to inform consumer risk communications regarding safe egg handling practices.

KEY WORDS: Consumer-phase risk assessment; domestic food handling and storage; *Salmonella enterica* serovar Enteritidis; sensitivity analysis; shell eggs

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1. INTRODUCTION

A *Salmonella enterica* serovar Enteritidis (SE) epidemic in the United States began in New England in 1978 and spread to much of the rest of the country in the next decade. The predominant source of SE is contaminated shell eggs. Despite national initiatives to control human salmonellosis caused by SE-contaminated eggs, the disease remains a national health concern. The most recent FoodNet data from the U.S. Centers for Disease Control and Prevention (CDC) indicates large, statistically significant reductions in most major foodborne pathogens;

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unfortunately, the prevalence of salmonellosis due to SE has not been significantly reduced since FoodNet baseline data collection began in 1996–1998.[1] Intervention programs have largely been focused at the production (farm) and processing phases. Voluntary state or industry sponsored egg quality assurance programs (EQAPs), based on the principles of hazard analysis and critical control points, have played a major role in reducing salmonellosis. A recent report indicates that a 1% increase in the number of eggs produced under an EQAP was associated with a 0.14% decrease in SE incidence.[2]

Although the number of food service and institutional outbreaks of salmonellosis appears to have declined due to the use of pasteurized eggs in pooled batches of eggs, CDC data suggest that domestic outbreaks and sporadic illnesses have increased.[3] Epidemiological studies have revealed a correlation between salmonellosis and consumer behaviors concerning egg handling and consumption.[4–6] However, the number of SE interventions focused at the consumer level has been relatively limited.

Morris summarized risky consumer factors associated with the transmission of SE by eggs as including poor refrigeration practices, improper storage of pooled eggs, use of raw eggs, time and temperature effects, and exposure of highly susceptible individuals.[7] Examples of unsafe egg consumption and handling practices at home include use of raw eggs, holding eggs and egg-containing food at room temperature, undercooking eggs and egg-containing foods, and pooling eggs.[3,8,9] There is clearly a need to understand how consumer behaviors concerning eggs and egg-containing products affect the risk of salmonellosis, and to determine whether control programs aimed at consumers would be effective at reducing salmonellosis incidence further.

The purpose of this study was to create a one-dimensional probabilistic model of the role of domestic storage and handling behaviors on salmonellosis risk associated with eggs and egg-containing food products. The model incorporates variability in inputs using updated information about consumer egg consumption and handling collected from a web-based consumer survey.[10] Subsequent analysis was used to evaluate the robustness of the risk estimates with respect to the assumptions made in the model, and to identify critical future research needs. Sensitivity analysis was used to identify consumer handling behaviors that contribute most to salmonellosis risk or that may be used as domestic control measures. What-if scenario analyses were used to identify possible control points for reducing the risk of salmonellosis associated with these food products.

2. MODEL DEVELOPMENT

2.1. Hazard Identification

According to CDC foodborne illness surveillance (FoodNet), *Salmonella* species remain one of the two leading causes of bacterial foodborne infection in the United States.[1] Responsible for an estimated 10% of foodborne illnesses, 26% of hospitalization, and 31% of deaths in the United States, nontyphoidal *Salmonella* is the leading cause of deaths and hospitalizations associated with known bacterial foodborne pathogens.[11] FoodNet surveillance in 2004 indicated an overall salmonellosis incidence of 14.7 per 100,000 persons, compared to 12.9 for *Campylobacter* and 5.1 for *Shigella*.[1]

Ranked after *Salmonella typhimurium*, SE has emerged as the second most common cause of salmonellosis.[1] Infection of laying hens with SE and the resultant contamination of eggs are believed to be the important sources of contamination that subsequently result in illness.[12–14] During 1990–2001, the U.S. state and territorial health departments reported 677 SE outbreaks, and among the 309 outbreaks with a confirmed vehicle of transmission, 241 (78.0%) were associated with shell eggs. The cost associated with human salmonellosis due to SE is estimated to range from $150 to $870 million annually.[15] The President's Council on Food Safety—Egg Safety Action Plan was formulated with the goal that egg-associated SE illness should be reduced by 50% by 2005 and eliminated by 2010.[8]

Two country-specific, farm-to-table risk assessments for SE illness associated with eggs were conducted by the Food Safety Inspection Service (FSIS)/U.S. Department of Agriculture (USDA)[15] and Health Canada,[16] and a worldwide farm-to-table model was developed by the World Health Organization (WHO) and Food and Agriculture Organization (FAO), based on the U.S. and Canadian models.[17] Risk assessments performed by other researchers evaluated the risk of SE illness associated with cracked eggs,[18] pasteurized liquid eggs,[19] egg storage and transportation,[20] egg production,[21] and egg consumption and handling behaviors at home.[7] The effects of time and temperature during egg collection, processing, transportation, and storage have also been evaluated.[20,22–24]

2.2. Exposure Assessment

In this study, the exposure to SE associated with the consumption of eggs and egg-containing foods following domestic storage and preparation was estimated for six food categories. In previous exposure assessments,[15–17] consumer preparation and handling behaviors were modeled largely by personal assumptions. In order to reduce uncertainty associated with consumer-related behaviors, several inputs (discussed later in the article) were informed by a national, web-based consumer survey including only participants who regularly purchased eggs (n = 1,076). Demographic details of survey participants are summarized elsewhere.[10]

A schematic diagram of the exposure assessment is given in Fig. 1. The model was coded in Matlab (The MathWorks, Natick, MA). The six food categories are summarized in Table I and further explained in Section 2.2.2. Inputs in the exposure assessment section include prevalence and initial contamination levels, consumer behaviors concerning egg handling and consumption, and the kinetic parameters for SE growth and inactivation. Table II summarizes the list of the inputs and their corresponding probability distributions.

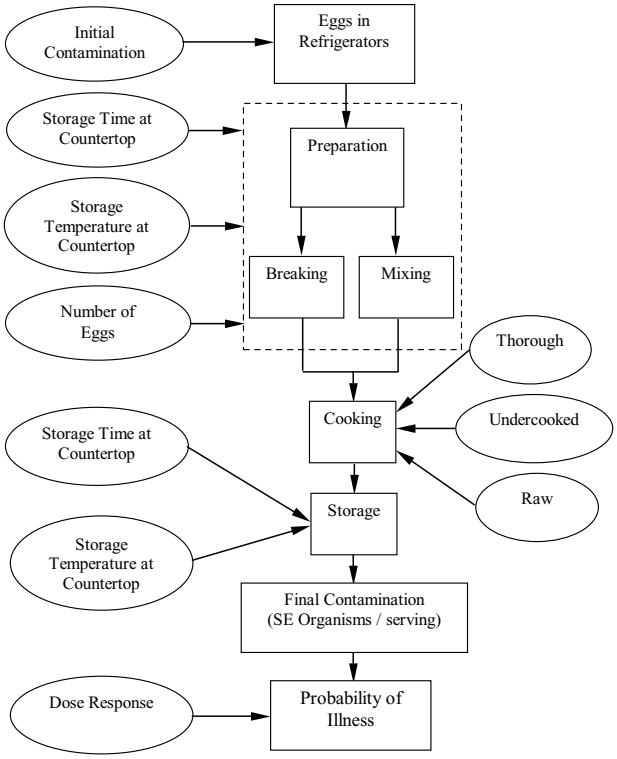

Fig. 1. Schematic diagram of the SE exposure model for domestic handling.

2.2.1. Initial Contamination

Shell eggs may be contaminated internally due to vertical transmission from the hen's ovaries to the egg yolk. To estimate the probability that an internally contaminated egg is used during meal preparation, first, the prevalence of internally contaminated shell eggs at home was specified as 1×10^{-5}, based on the annual incidence of SE-contaminated eggs.[25] Next, the total number N of eggs used during meal preparation was modeled based on data from our consumer behavior survey;[10] the probability that n contaminated eggs are selected for a single food preparation that includes N eggs was modeled as a binomial distribution. The level of SE per contaminated egg was based on a farm-to-table exposure model for the level of SE in raw shell eggs due to vertical transmission.[20] The exposure model output, expressed as a probability distribution ($\log_{10}$ SE CFU/egg) for eggs stored in the consumer household, was used as the initial contamination level input for this model.

Most shell eggs are not internally contaminated.[25] However, egg-containing foods may be contaminated with SE located externally on the shell. Although SE organisms on eggshells die rapidly, their survival is enhanced by high relative humidity and low temperature during storage.[26,27] Limited data show that the prevalence of SE contamination on eggshells ranges between 0% and 19%.[28–31] A Pert (0.001, 0.1, 0.2) distribution was used to account for variability in the prevalence of SE contamination on eggshells. The number of SE cells transferred during food preparation from an individual contaminated eggshell to the egg contents (CFU/egg) was expected to vary between 0 and 20 with a uniform distribution.[32]

2.2.2. Consumer Preparation and Handling

In order to evaluate the impact of consumer preparation and handling on levels of SE in egg-containing foods, the foods were classified into six categories (Table I) representing combinations of three key preparation and handling behaviors, i.e., pooling of eggs, the use of the egg (as an egg dish or as an ingredient), and the degree of cooking. These three consumer behaviors have been shown to impact the final number of SE cells in the food at consumption.[15–17]

More eggs become contaminated when non-contaminated and contaminated eggs are pooled.

Table I. Characterization of Food Categories Considered in the SE Model

Category	Pooling	Egg/ Ingredient	Example Foods	Consumer Survey[a]	
				Percentage of Population Cooking Thoroughly[b]	Percentage of Total Eggs Consumed
I	No	Egg	Fried eggs	51%	31%
II	No	Egg	Soft-boiled, hard-boiled, poached eggs	83%	19%
III	Yes	Egg	Scrambled eggs, omelettes	98%	35%
IV	Yes	Ingredient	Ice cream, eggnog, Caesar salad dressing, raw cookie dough	73%	1%
V	Yes	Ingredient	Custard, egg soup, soufflé, lasagna	98%	5%
VI	Yes	Ingredient	Bread, cake, muffins, cookies, waffles	98%	9%

[a]Results from consumer survey ($n = 1{,}076$).
[b]The percentage of eggs that are undercooked = 100% − percentage thoroughly cooked for Cats. I–III and V–VI. For Cat. IV, 26% of subjects reported using raw eggs, and the percentage of undercooked was 1.

Pooling eggs has been recognized as a major risk factor associated with SE outbreaks in food service and institutions.[3,8] However, the effect of pooling is relatively minor during domestic handling because of the limited number of eggs used in a single instance of food preparation. Furthermore, when multiple servings are prepared at the same time, pooling has a dilution effect with respect to the levels of SE contamination per serving. Pooling was assumed for foods in Categories III–VI, e.g., scrambled eggs and in foods for which eggs were used as an ingredient. The number of eggs pooled for a single food preparation event was modeled using a discrete distribution based on the consumer behavior survey.[10]

Table II. Input Variables and Corresponding Probability Distributions in the SE Model

Inputs	Distribution	Source
Initial contamination (log CFU/egg)	Log-normal (2.00, 0.59)	Latimer *et al.* (2002)
Egg shell prevalence	Pert (0.001, 0.01, 0.2)	Expert judgment
SE from contaminated eggshell (CFU)	Uniform (0, 20)	Expert judgment
Number of eggs mixed	Discrete (n, p)[a]	Consumer survey[b]
Storage temperature before cooking (°C)	Pert (4, 10, 21)	Expert judgment
Storage time before cooking (hours)	Uniform (0, 2)	Expert judgment
Storage temperature after cooking (°C)	Pert (4, 21, 35)	Expert judgment
Storage time after cooking (hours)	Weibull (0.74, 0.70)[c]	Consumer survey
Portion of eggs (Category I)	Discrete (n, p)[d]	CSFII, 1994–1996, 1998
Portion of eggs (Categories II to VI)	Log-normal (μ, δ)[e]	CSFII, 1994–1996, 1998
Cooking reduction (thoroughly cooked)	Uniform (6, 8)	FSIS/USDA, 1998
Cooking reduction (undercooked)	Pert (α, β, γ)[f]	FSIS/USDA, 1998
Serving size (g) (Category I)	Min{Log-normal (70.15, 42.04), 283}	CSFII, 1994–1996, 1998
Serving size (g) (Category II)	Min{Log-normal (44.15, 77.97), 263}	CSFII, 1994–1996, 1998
Serving size (g) (Category III)	Min{Log-normal (75.91, 62.11), 410}	CSFII, 1994–1996, 1998
Serving size (g) (Category IV)	Min{Log-normal (16.14, 12.58), 382}	CSFII, 1994–1996, 1998
Serving size (g) (Category V)	Min{Log-normal (12.656, 4.456), 143}	CSFII, 1994–1996, 1998
Serving size (g) (Category VI)	Min{Log-normal (10.04, 14.50), 169}	CSFII, 1994–1996, 1998

[a]One egg is used for Categories I and II. Category III: $n = \{1, 2, 3, 4, 5, 6, 7, 8\}$; $p = \{0.09, 0.17, 0.21, 0.20, 0.15, 0.09, 0.05, 0.02\}$. Categories IV to VI: $n = \{1, 2, 3, 4, 5, 6, 7\}$; $p = \{0.13, 0.21, 0.23, 0.19, 0.12, 0.07, 0.03\}$.
[b]Results from a web-based consumer survey ($n = 1{,}076$).
[c]The distribution was truncated at 24 hours for foods in Category IV. For foods in other categories, the distribution was truncated at six hours.
[d]$n = \{$Log-normal (0.37, 0.07), Log-normal (0.95, 0.01)$\}$; $p = \{0.19, 0.81\}$ The distribution is truncated between 0 and 1.
[e]Category II: $\mu = 0.318$, $\delta = 0.558$; Category III: $\mu = 0.558$, $\delta = 0.149$; Category IV: $\mu = 0.329$, $\delta = 0.733$; Category V: $\mu = 0.117$, $\delta = 0.125$; Category VI: $\mu = 0.135$, $\delta = 0.101$; The distributions are truncated between 0 and 1.
[f]Category I: $\{\alpha, \beta, \gamma\} = \{0, 4, 7\}$; Category II: $\{\alpha, \beta, \gamma\} = \{0, 1, 7\}$; Categories III to VI: $\{\alpha, \beta, \gamma\} = \{0, 6, 7\}$.

Eggs may be used as eggs or as ingredients within foods. Categories I, II, and III considered eggs as egg dishes; Categories IV, V, and VI considered eggs as ingredients. Eggs used for the preparation of egg dishes comprised nearly 100% of the entire food product; when eggs were used as ingredients, they comprised a variable amount of the total food content, ranging from 5% to 85%, depending on the category.[33,34] Typically, a log-normal distribution was considered for the portion of eggs used in each food category. Each distribution was truncated between 0 and 1.

For the cooking step, three possibilities were modeled: thorough cooking, undercooking, and no cooking. Data from our consumer behavior survey[10] was used to determine the frequency with which foods are prepared, according to each of the three alternatives. For example, 51% of respondents reported thoroughly cooking foods in Category I and 98% of respondents reported thoroughly cooking foods in Category VI. Next, the effectiveness ($\log_{10}$ SE reduction) of each alternative was modeled. For all foods, thorough cooking was modeled to reduce between 6 and 8 $\log_{10}$ of SE.[15] The effect of undercooking varied for different categories (Table II). For example, partial cooking for foods in Category I was expected to result in $\log_{10}$ reductions varying between 0 and 7 following a Pert distribution, with the most likely value of 4; however, undercooking of foods in Category II was most likely to result in a one-$\log_{10}$ reduction.[15] Foods in Category IV were most likely to contain raw egg as an ingredient.[16] Approximately 25% of respondents reported preparing Category IV foods with uncooked eggs. No reduction in SE was modeled for uncooked foods.

SE organisms were expected to be capable of growth during countertop storage and preparation prior to cooking. After the cooking step, the growth of surviving SE cells in the prepared food was modeled. Multiple predictive microbial growth models for *Salmonella* have been developed,[35–38] including functions such as the Gompertz equation,[38] the exponential growth rate model,[16] and the response surface model.[39] For this study, the latter model is used for estimation of growth.[20] The response surface model is based on previously reported growth kinetic data for *S. typhimurium*,[39] and was used as a surrogate for SE growth in this study. The model can be mathematically expressed as:

$$\lambda = \exp(a_0 + a_1(\mathrm{NaCl}) + a_2 \times T + a_3(\mathrm{NaCl} \times T) + a_4(\mathrm{NaCl} \times \mathrm{NaCl}) + a_5 \times T^2) \quad (1)$$

$$\mu = \exp(b_0 + b_1(\mathrm{NaCl}) + b_2 \times T + b_3(\mathrm{NaCl} \times T) + b_4(\mathrm{NaCl} \times \mathrm{NaCl}) + b_5 \times T^2), \quad (2)$$

where

λ = lag time (hours);
μ = growth rate ($\log(\frac{\mathrm{CFU}}{\mathrm{egg} \times \mathrm{hr}})$);
NaCl = the concentration of sodium chloride, 0.5% for the yolk contents;
T = storage temperature (°C);
a_i = parameter estimates: $a_0 = 5.911$, $a_1 = -0.2013$, $a_2 = -0.2754$, $a_3 = -0.0013$, $a_4 = 0.0333$, $a_5 = 0.0033$;
b_i = parameter estimates: $b_0 = -6.2251$, $b_1 = -0.0114$, $b_2 = 0.3234$, $b_3 = 0.0020$, $b_4 = -0.0085$, $b_5 = -0.0045$.

The duration of food preparation, which was assumed to take place at room temperature, was assumed for all categories to range with equal probability between zero and two hours. To estimate the countertop storage time after cooking, a discrete probability distribution was developed from self-reported consumer behaviors. For foods in Categories I, II, and III, the maximum storage time was truncated at six hours. For foods in Categories IV and V, the maximum storage time was truncated at 24 hours in order to take into account the extra storage time for leftovers. For foods in Category VI, growth during countertop storage was not modeled, as these products have low water activity and would be unlikely to support the growth of the pathogen.

Prior to preparation, the eggs were assumed to be stored in the refrigerator,[40] and no growth was modeled. During meal preparation, the temperature of the eggs was assumed to range between 4°C (directly from the refrigerator) and 21°C (room temperature). The most likely temperature during preparation was assumed to be 10°C. After cooking, the temperature of the foods was assumed to range between 4°C (refrigeration temperature) and 35°C, with the most likely temperature 21°C (room temperature). Countertop storage temperatures lower than room temperature were considered for eggs prior to cooking, because it was assumed that the eggs were taken out from the refrigerator not long before the cooking process. Consistent with reported data, no growth was modeled for eggs stored at temperatures below 10°C.[41–43] The maximum level of SE was limited to 9.5 $\log_{10}$ CFU/egg[20] and 8 $\log_{10}$ CFU/g food for countertop storage before cooking and after cooking, respectively, considering that foods with more

contamination would be discarded because of obvious signs of spoilage.[44]

2.3. Dose Response

The dose-response relationship used in the SE model is based on the relationship suggested by WHO/FAO.[17] The model has a Beta-Poisson functional form as:

$$P_{\text{ill}} = 1 - \left(1 + \frac{\text{Dose}}{5587}\right)^{-0.4047} \quad (3)$$

where,

P_{ill} = probability of illnesses
Dose = intake of Salmonella (CFU/serving).

Probability of illness per serving for each of the six food categories is estimated. The relative consumption proportions for each food category were used to aggregate individual probabilities in order to estimate the overall probability of illness from consumption of eggs and egg-containing foods per serving.

3. MODEL ANALYSIS

In order to estimate the probability of SE illness resulting from consumption of eggs and egg-containing food on a per-serving basis, Monte Carlo simulation was used and probability distributions of inputs were propagated through the model. The number of iterations for all simulations was 10,000. Each iteration represented a single possible egg handling scenario. The Latin Hypercube sampling technique was used to sample from probability distributions of inputs.

3.1. Bootstrap Simulation for Quantification of Uncertainty

Uncertainties in the hazard, exposure, and dose-response information may result in unrealistic risk estimates. Sources of uncertainty can include problem and scenario specification, model uncertainty, sampling error, lack of representativeness, lack of empirical basis, and disagreement of experts.[45] The first two sources of uncertainty are related to structural uncertainty, while the rest are related to uncertainty in model inputs. Probability distributions of model inputs are typically based on analysis of available data. Typically, parameters of those distributions (e.g., geometric standard deviation of a log-normal distribution) are estimated using relatively small sets of sample data. Thus, there is uncertainty in the estimates of these statistics due to sampling error. Quantification of sampling error may be done using classical statistical techniques or numerical simulation methods. We used bootstrap simulation to quantify uncertainty due to sampling error in different percentiles of the estimated risk of SE illness. Bootstrap simulation is a numerical technique originally developed for the purpose of estimating confidence intervals for statistics.[46] Typically, bootstrap simulations are repeated a number of times to evaluate numerical stability of the output distribution, by comparing results among the multiple bootstrap simulations.

Bootstrap simulation uses a conceptually straightforward approach. In the case of the SE model, a random sample, referred to as the "bootstrap sample," was generated from each of the probability distributions developed or assumed for inputs. The maximum likelihood estimation (MLE) approach was used to fit a probability distribution to each of the bootstrap samples. For example, for the initial contamination with a log-normal distribution, the MLE approach was used to fit a new log-normal distribution to the corresponding bootstrap sample. The parameters of the new distribution differ from those for the original distribution, representing uncertainty due to sampling error.

The number of bootstrap replications required depends upon the information needed. For example, to calculate the standard error of a statistic, Efron and Tibshirani[46] suggest 200 or less bootstrap replications. However, for estimation of confidence intervals, more replication may be required; we considered 200 bootstrap replications to be satisfactory for this study.

3.2. Sensitivity Analysis for Identification of Key Sources of Uncertainty

In order to prioritize data collection activities, it is useful to identify the key sources of uncertainty. Because uncertainty results from lack of knowledge and specifically, as addressed in this article, from lack of proper and representative data, the collection of additional data is the only viable method for reducing uncertainty. In many cases, the uncertainty in the model output may be influenced by only a subset of the model inputs and their corresponding assumptions, also known as key sources of uncertainty. It would be an unwise allocation of scarce resources to spend an equal amount of effort collecting data and developing probability distributions for all model inputs if the output is sensitive to only a small number of inputs.

The key sources of uncertainty for each food category were separately identified for the mean, 95th, and 99th percentiles of the probability of SE illness. Spearman correlation coefficients[47–49] were used to identify the key sources of uncertainty. Spearman correlation coefficients evaluate the strength of nonlinear but monotonic association between paired rank transformed input and output values. Inputs were ranked based upon the relative magnitude of statistically significant Spearman correlation coefficients with a significance level of 5%.

3.3. Sensitivity Analysis for Identification of Key Sources of Variability

Knowledge of key sources of variability can guide the identification of significant subpopulations that merit more focused study, or the targeting of risk management strategies to controllable sources of variation. The choice of a sensitivity analysis method depends on the characteristics of the model.[50] Typical characteristics of quantitative microbial food safety process risk models are nonlinearity, interaction between inputs, thresholds, and saturation points in the model response, and use of both categorical and continuous inputs.[51] An ideal sensitivity analysis method is independent from assumptions about the model structure.[52] Specifically, a sensitivity analysis method should not require any assumptions regarding the functional form of the risk model and should be applicable to different model formulations.

Sobol's method[52,53] can cope with both nonlinear and nonmonotonic models and does not assume any functional form for the model. Sobol's method provides a truly quantitative ranking of inputs and not just a relative qualitative measure.[54] The types of influences on an input that are captured by Sobol's method include those that are additive, nonlinear, and/or with interactions. Sobol's method has been used for sensitivity analysis of computationally complex models;[52,55] however, we believe this is the first application of Sobol's method in the field of quantitative microbial risk assessment. We selected Sobol's method for identification of key sources of variability because of its unique advantages as compared to the typical sensitivity analysis techniques such as regression-based methods. These advantages are further illustrated in the results and discussion sections.

The main idea behind Sobol's method is the decomposition of the function $f(x)$ including k inputs into summands of increasing dimensionality:[54]

$$f(x_1, \ldots, x_k) = f_0 + \sum_{i=1}^{k} f_i(x_i) + \sum_{i=1}^{k} \sum_{j=i+1}^{k} f_{ij}(x_i, x_j) + \cdots + f_{1,2,\ldots,k}(x_1, \ldots, x_k). \quad (4)$$

The total variance D of $f(x)$ and the partial variances from each of the terms in Equation (4) are computed as:

$$D = \int f^2(x)\, dx - f_0^2 \quad (5)$$

$$D_{i_1,\ldots,i_s} = \int_0^1 \cdots \int_0^1 f_{i_1,\ldots,i_s}(x_1, \ldots, x_s)\, dx_{i_1} \ldots dx_{i_s}, \quad (6)$$

where $1 \le i_1 < \ldots < i_s \le k$, $s = 1,\ldots, k$, and k is the number of inputs. By squaring and integrating Equation (4) over the k-dimensional input space, we have:

$$D = \sum_{i=1}^{k} D_i + \sum_{i=1}^{k} \sum_{j=i+1}^{k} D_{ij} + \cdots + D_{1,2,\ldots,k}. \quad (7)$$

Thus, a sensitivity measure $S(i_1,\ldots,i_s)$ is defined as

$$S_{i_1,\ldots,i_s} = \frac{D_{i_1,\ldots,i_s}}{D} \quad \text{for} \quad 1 \le i_1 < \ldots < i_s \le k, \quad (8)$$

where S_i is called the first-order sensitivity index for input x_i, which measures the main effect of x_i on the output representing the fractional contribution of x_i to the variance of $f(x)$. S_{ij}, for $i \neq j$, is called the second-order sensitivity index, which measures the interaction effect between x_i and x_j. The interaction effect is the part of the variation in $f(x)$ due to x_i and x_j that cannot be explained by the sum of the individual effects of x_i and x_j. The decomposition in Equation (7) has the useful property that all the terms in Equation (8) sum to 1; that is,

$$\sum_{i=1}^{k} S_i + \sum_{1 \le i < j \le k} S_{ij} + \ldots + S_{1,2,\ldots,k} = 1. \quad (9)$$

Sobol's method can also provide insight regarding the total effect of each input. The total effect of an input, which includes both the main effect as well as interaction effects of any dimensionality, is defined as the sum of all the sensitivity indices involving that input.[53,56] For example, if there are three inputs x_1, x_2, and x_3, the total effect of x_1 is given by $S(x_1) + S(x_1 \times x_2) + S(x_1 \times x_2 \times x_3)$, where $S(i)$ is the sensitivity index of the term i. Thus, the total effect of x_i can be estimated as:

$$\mathrm{TS}_i = S_i + S_{i(\sim i)} = 1 - S_{\sim i}, \quad (10)$$

where $S_{\sim i}$ is the sum of all the $S_{i_1,\ldots,i_s}$ terms that does not include the index i, i.e., the total fractional variance complement to input x_i, $D_{\sim i}$. Thus, the total contribution of input x_i to the total output variation is given by:

$$\mathrm{TS}_i = 1 - \frac{D_{\sim i}}{D}.$$

Note that the total effects of inputs do not provide a complete characterization of the sensitivity. However, the total effects are much more reliable than the first-order (main effects) indices in order to investigate the overall effect of each single input on the output.

The algorithm for the estimation of main and total effects associated with each input was coded using Matlab (The Mathworks, Natick, MA).

3.4. What-If Scenario Analysis

What-if scenarios were conducted for inputs that were identified as key sources of variability. For each what-if scenario analysis, the selected input was varied and the resulting changes in the model outputs were collected. For example, countertop storage time was varied between 0 and 48 hours. Within each simulation, the values of other input variables were randomly sampled from their corresponding distributions with a total of 10,000 iterations.

4. RESULTS

4.1. Probabilities of SE Illness for Different Food Categories

The first step in the SE risk assessment was to estimate the illness from consumption of eggs and egg-containing foods (illness/serving). Considering a binomial distribution for the number of contaminated eggs, the probability associated with the number of contaminated eggs depends on the total number of eggs used in a single serving. For example, if one egg is used, the probability that a contaminated egg is selected is 5×10^{-5} (data not shown). This probability is higher if more than one egg is used in a serving. Our consumer survey indicated that typically 96% to 98% of foods in Categories III to VI (e.g., omelettes, baked foods, breads) are prepared with fewer than 10 eggs.[10] Thus, for those food categories, the probability of using an SE-contaminated egg varies between 5×10^{-4} and 5×10^{-5}. Because of the low probability of selecting an SE-contaminated egg, typically 99.9% to 99.99% of food preparation scenarios in a Monte Carlo simulation were identified as having no contamination. To avoid overwhelmingly large numbers of iterations with no (zero) contamination, we only modeled food preparations containing at least one contaminated egg.

Table III summarizes the estimated illness from consumption of eggs and egg-containing foods (illness/serving) prepared using SE-contaminated eggs. The mean estimated risk is as high as 2.05×10^{-4} for Category IV (home-made ice cream) and as low as 2.59×10^{-10} for Category VI (baked foods). The actual probability of illness from consumption of eggs and egg-containing foods is expected to be 4 to 5 logs lower if preparation with no contaminated eggs is taken into account. Considering the mean probability of illness when SE-contaminated eggs are used in domestic food preparation, the ranking of the six food categories for the risk of SE illness is: (1) Category IV (home-made ice cream); (2) Categories I and II (fried eggs and boiled eggs; (3) Category III (omelettes); and (4) Categories V and VI (baked foods and breads). Risks for foods in Categories I and II and for foods in Categories V and VI are considered to be comparable.

4.2. Quantification of Uncertainty

The results of 200 bootstrap replications are summarized in Table IV for the mean, 50th, 75th, 95th, and 99th percentiles of the distribution. Typically, the bootstrap results showed a wide range of uncertainty

Food Category	Mean	50th Percentile	75th Percentile	95th Percentile	99th Percentile
I	7.52×10^{-5}	0	0	5.65×10^{-5}	7.71×10^{-4}
II	7.07×10^{-5}	0	0	3.48×10^{-5}	7.14×10^{-3}
III	1.64×10^{-8}	0	0	0	0
IV	2.05×10^{-4}	0	1.78×10^{-6}	4.16×10^{-4}	2.8×10^{-3}
V	1.26×10^{-10}	0	0	0	0
VI	2.59×10^{-10}	0	0	0	0
Overall	4.87×10^{-5}	0	0	0	4.05×10^{-4}

Table III. Estimated Illness from Consumption of Eggs and Egg-Containing Foods (Illness/Serving) Prepared Using at Least One Contaminated Egg

Table IV. Uncertainty in the form of 95% Probability Range of Values for Mean, 50th, 75th, 95th, and 99th Percentiles of the Probability Distribution for SE Illness

Food Categories	Mean	50th Percentile	75th Percentile	95th Percentile	99th Percentile
I	$(5.4 \times 10^{-5}, 1.6 \times 10^{-3})$	0.0	0.0	$(0.0, 4.5 \times 10^{-4})$	$(2.6 \times 10^{-4}, 1.7 \times 10^{-2})$
II	$(5.6 \times 10^{-5}, 2.4 \times 10^{-3})$	0.0	0.0	$(2.5 \times 10^{-5}, 1.2 \times 10^{-3})$	$(1.3 \times 10^{-5}, 7.0 \times 10^{-4})$
III	$(0.0, 9.28 \times 10^{-7})$	0.0	0.0	0.0	0.0
IV	$(3.4 \times 10^{-5}, 6 \times 10^{-2})$	0.0	$(0.0, 1.9 \times 10^{-4})$	$(1.2 \times 10^{-4}, 2.1 \times 10^{-3})$	$(3.4 \times 10^{-5}, 5.0 \times 10^{-2})$
V	$(0.0, 3.1 \times 10^{-8})$	0.0	0.0	0.0	0.0
VI	$(0.0, 4.4 \times 10^{-8})$	0.0	0.0	0.0	0.0
Overall	$(2.1 \times 10^{-5}, 1.7 \times 10^{-3})$	0.0	0.0	$(0, 3.4 \times 10^{-5})$	$(2.4 \times 10^{-4}, 7.5 \times 10^{-3})$

in estimates of the probability of SE illness for the mean and selected percentiles. However, for some food categories and for selected percentiles, there was no uncertainty range. For example, while there was no uncertainty in estimates of the 50th and 75th percentiles of risk for foods in Category II (e.g., boiled eggs), the mean probability of illness for this category had a 95% probability range between 5.6×10^{-5} and 2.4×10^{-3}. Considering the range of uncertainty associated with the mean probability of SE illness, the ranking of the six food categories from the greatest uncertainty to the least was as follows: (1) Category IV (home-made ice cream); (2) Categories I and II (fried eggs and boiled eggs; (3) Category III (omelettes); and (4) Categories V and VI (baked foods and breads). Thus, the mean probability of SE illness due to consumption of foods in Category IV had the highest uncertainty. In contrast, foods in Category V had the lowest uncertainty with respect to the mean probability of SE illness per serving. The ranking of different food categories based on the magnitude of uncertainty associated with mean probability of illness is similar to the ranking based on the magnitude of risk (Section 4.1), i.e., food categories with higher risk of illness have larger uncertainty associated with the risk. However, this finding is specific to our model and it may not be the same for other risk assessment models.

4.3. Identification of Key Sources of Uncertainty

The key sources of uncertainty for each food category were separately identified for mean, 95th, and 99th percentiles of the probability of SE illness using Spearman correlation coefficients (Fig. 2). Results shown in Fig. 2 are consistent with those given in Table IV in the sense that for food categories that have uncertainty ranges for mean and selected percentiles, key sources of uncertainty and their corresponding correlation coefficients are given. For example, all food categories had uncertainty ranges associated with their mean probability of illness. However, only foods in Categories I, II, and IV had uncertainty ranges associated with the 95th and 99th percentiles of the probability of illness.

The key sources of uncertainty for each food category were different for the mean and selected percentiles of the probability of illness. However, initial contamination, storage time on countertops after cooking, and log reduction due to cooking were typically among the key sources of uncertainty for the mean and the selected percentiles. For example, for foods in Category IV, which had the highest probability of SE illness, countertop storage time and temperature after cooking, $\log_{10}$ reduction in contamination due to cooking, and portion of eggs used in foods were key sources of uncertainty for the mean probability of SE illness per serving. However, for the 95th percentile, the initial level of contamination was a major source of uncertainty. Additional sources of uncertainty for this percentile included proportion of eggs used as ingredients and storage time after cooking. At the 99th percentile of risk of SE illness, key sources of uncertainty were storage time after cooking, proportion of eggs used as ingredients, and $\log_{10}$ reduction due to cooking.

4.4. Identification of Key Sources of Variability

Main effects and total effects of inputs based on the Sobol's method are shown in Fig. 3 for foods in Categories I, II, and IV. These food categories had a relatively higher mean risk of SE illness on a per-serving basis. All model inputs were analyzed for their contribution to the output variance; however, only inputs that made a substantial contribution to the

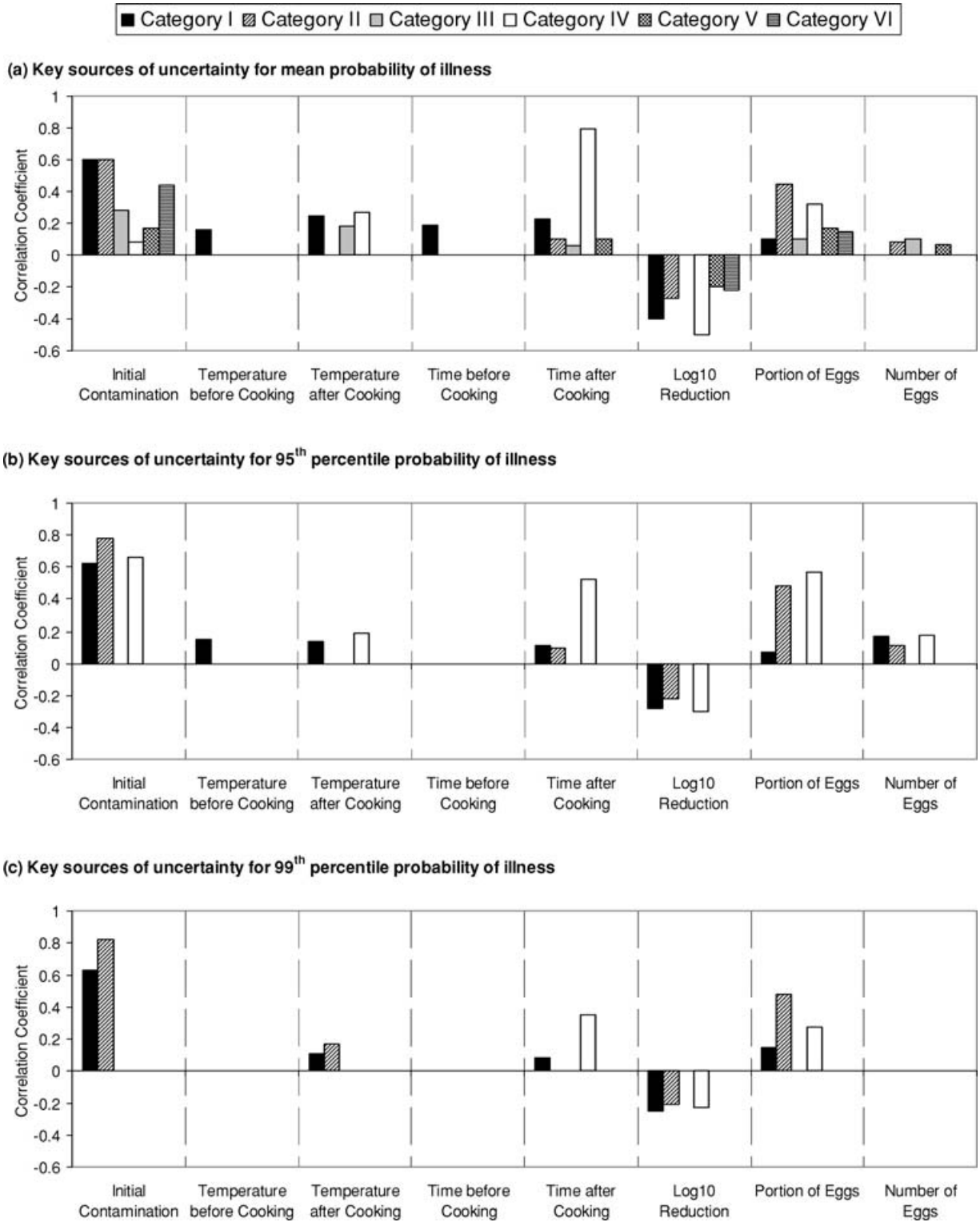

Fig. 2. Key sources of uncertainty for: (a) mean; (b) 95th percentile; and (c) 99th percentile of the probability of SE illness in different food categories.

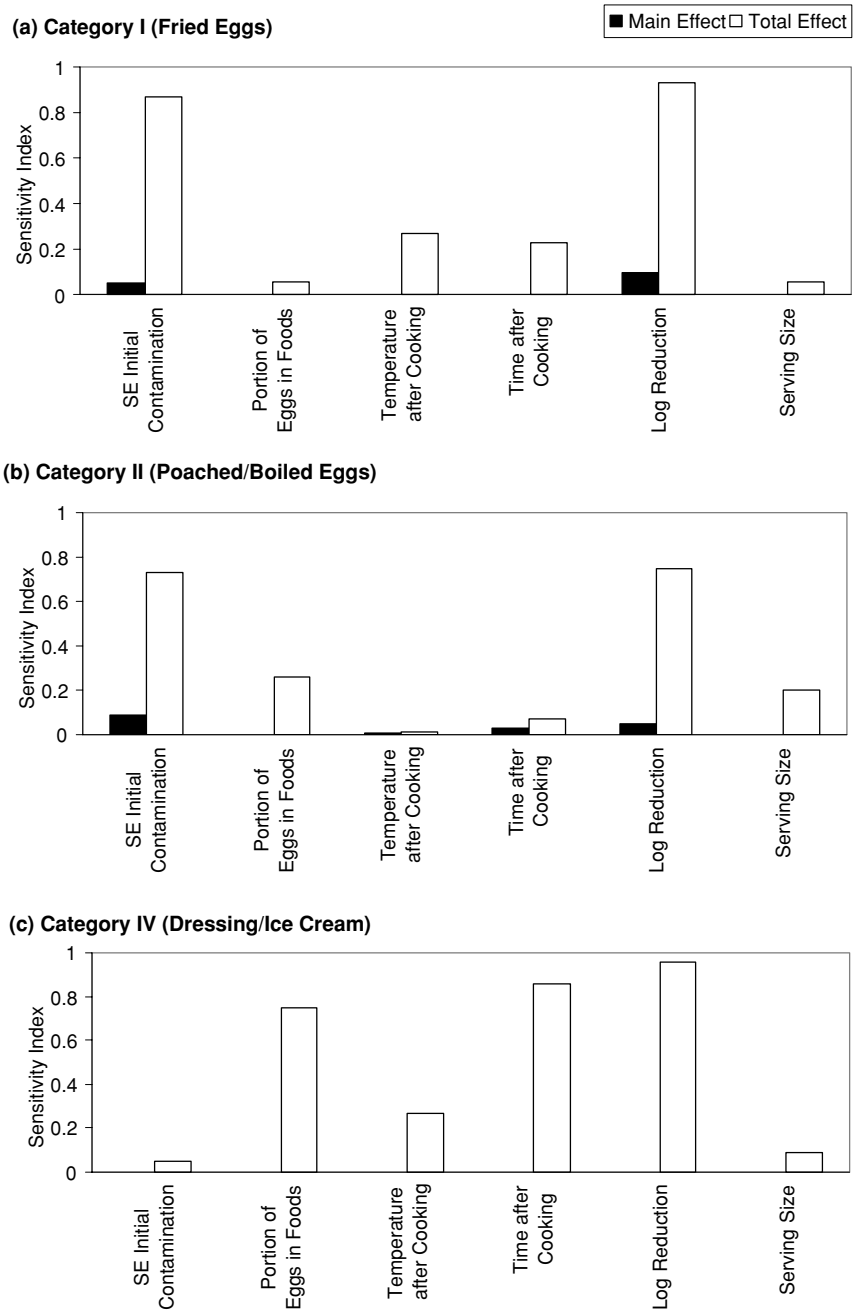

Fig. 3. Main effect and total effect of key sources of variability based on Sobol's methods for: (a) fried eggs (Category I); (b) poached/boiled eggs (Category II); and (c) dressing/ice cream (Category IV).

output variance either in the form of their main effects or total effects are shown.

Fig. 3 shows that inputs typically did not have substantial main effects and that their contributions to the output variance were mostly due to their total effects, especially interactions between inputs. For example, the summation of main effects for inputs in Fig. 3a (Category I—fried eggs) was only 0.15; thus, only 15% of the output variance was attributed to the linear effects of the inputs. For foods in Category II (e.g., boiled eggs), 17% of the output variance was apportioned to main effects of the inputs (Fig. 3b), and for foods in Category IV (e.g., home-made salad dressings and ice cream), no input had a significant main effect (Fig. 3c). However, some inputs substantially affected the output via their interaction effects. For example, 82% of the output variance for foods in Category I (Fig. 3a) was due to the interaction between the initial level of contamination and other inputs.

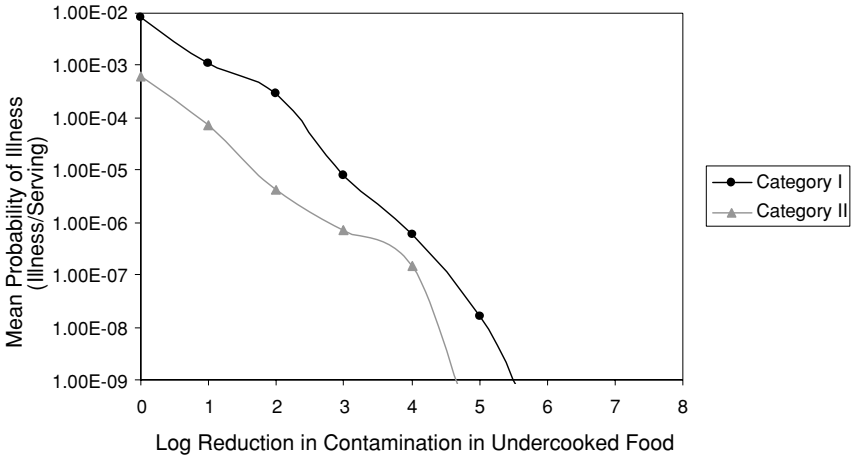

Fig. 4. Variation of mean probability of SE illness with respect to the log reduction in undercooked foods for foods in Categories I and II.

Important inputs based on the relative magnitude of the total sensitivity indices were $\log_{10}$ reduction due to cooking, initial level of SE contamination, storage time and temperature after cooking, the proportion of egg used as an ingredient in the food, and the serving size. For foods in Categories I and II, the initial level of contamination and the $\log_{10}$ reduction associated with cooking had relatively high total effects. Thus, reducing the initial level of contamination as well as targeting consumer education on thorough cooking would be most effective at reducing illnesses associated with eggs that are fried, boiled, and poached. Meanwhile, countertop storage conditions after preparation (i.e., time and temperature) were relatively more important for foods in Category IV than in Categories I and II. Results of our consumer survey indicated that 26% of servings for recipes such as dressings and ice cream may include uncooked eggs. Thus, in order to reduce illness associated with foods containing raw eggs, foods should be refrigerated immediately after preparation to control the growth of SE cells in the food.

4.5. What-If Scenario Analysis

What-if scenario analysis is useful for identifying possible control points for reducing the risk of salmonellosis associated with the consumption of eggs and egg-containing products. Scenarios were conducted only for inputs that were identified as key sources of variability in Section 4.4, including $\log_{10}$ reduction in contamination in undercooked foods, SE level in contaminated eggs, and countertop storage time after cooking. Two of these inputs can be directly impacted via consumer advisories and recommendations.

Fig. 4 shows the variation in the mean probability of SE illness with respect to the $\log_{10}$ SE reduction in undercooked foods in Categories I and II. Log_{10} reduction in SE for thoroughly cooked foods are likely to be 6 to 8 logs.[15] However, our consumer survey indicated that a significant proportion of foods in these two categories may be undercooked, which is associated with lower $\log_{10}$ reductions.[10] The what-if scenario analysis indicated that when there was more than 4.5 and 5.5 $\log_{10}$ (CFU/g) reduction in SE contamination for foods in Categories I and II, respectively, the mean probability of SE illness was limited to 10^{-9}. Additional research is required to characterize the degree of cooking that would be required to reach these reduction levels.

Fig. 5 shows the variation in the mean probability of SE illness with respect to the initial level of SE in contaminated eggs for all food categories. On a log-log scale, foods in Categories I, II, and IV showed approximately linear responses to variation in initial contamination level. Some servings in these food categories may be undercooked or uncooked; even with initial contamination of 1 CFU/egg, the mean probability of SE illness did not decrease to less than a level of approximately 10^{-8}. Foods in Categories III, V, and VI showed less sensitivity to low values of initial contamination because most of the servings in these categories were thoroughly cooked. Limiting the initial contamination in eggs to 0.5 and 2 $\log_{10}$ (CFU/egg) can control the risk to a low value of approximately 10^{-18} for foods in Category III, and Categories V and VI, respectively.

Sensitivity analysis results indicated that countertop storage time was an important source of variability for foods in Categories I, II, and IV. All three categories had approximately similar responses when

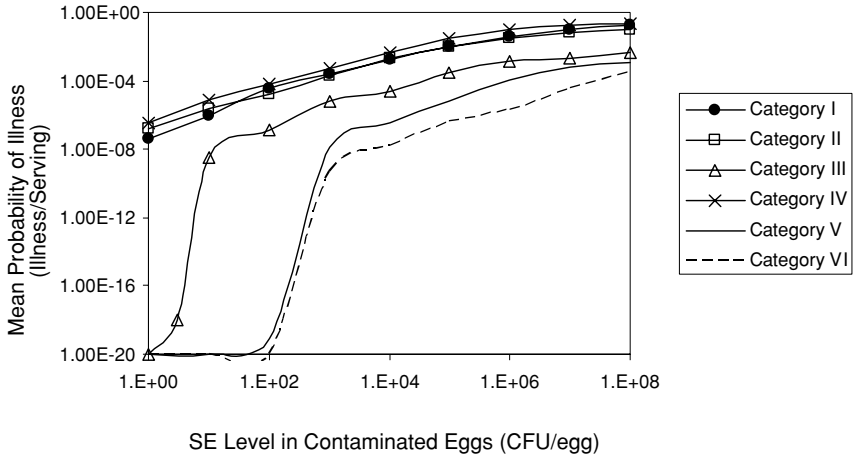

Fig. 5. Variation of mean probability of SE illness with respect to the SE level in contaminated eggs for the six food categories.

storage time was varied between 0 and 48 hours (Fig. 6). Reducing the countertop storage time after cooking appeared to be less effective at reducing risk than reducing the initial contamination or increasing the $\log_{10}$ reduction that would occur as a consequence of thorough cooking. What-if scenario analysis of countertop storage time showed that the risk for foods in these categories substantially increased if servings were kept at room temperatures for more than eight hours, which is an unlikely practice.

5. DISCUSSION AND CONCLUSIONS

Although previous farm-to-table risk assessment models for SE illness associated with the consumption of eggs have been developed, risk associated specifically with home and consumer behaviors has not received much attention. We developed a one-dimensional probabilistic home-phase risk assessment model for SE in eggs and egg-containing products that considered variability in inputs using updated information about consumer egg consumption and handling practices collected from a nationally representative web-based survey of 1,076 consumers.[10]

As perhaps expected, our model identified Category IV foods, such as home-made salad dressings and ice cream, as having the highest probability of SE illness on a per-serving basis. This is largely because raw eggs are a relatively common ingredient in these foods, which are subsequently served either uncooked or only lightly cooked; furthermore, these foods may be stored at room temperature on a countertop for an extended period of time after cooking. However, it is important to recognize that the risk estimate for foods in Category IV also manifested the greatest uncertainty associated with choice and parameterization of input distributions. The salmonellosis risks associated with the consumption of Category I and II foods, i.e.,

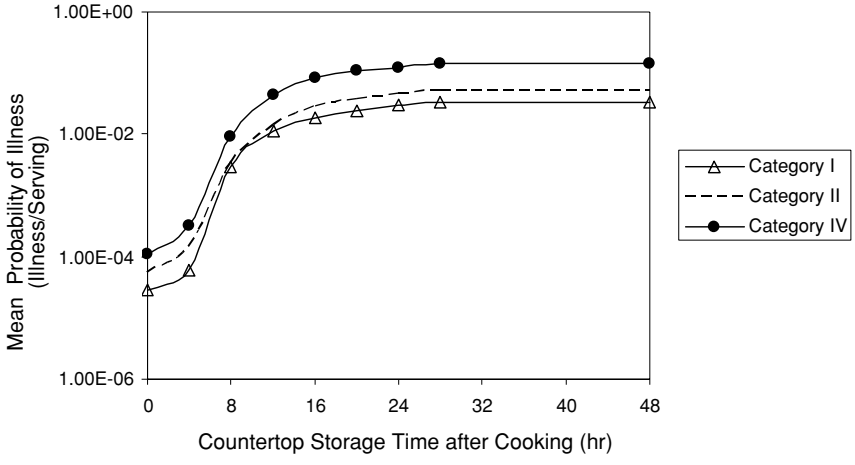

Fig. 6. Variation of mean probability of SE illness with respect to the countertop storage time after cooking.

fried, poached, or boiled eggs, were comparable and most likely due to undercooking of intact egg yolks. Category VI foods, for example, breads, had the lowest risk of SE contamination and subsequent disease. Foods in this category are well-cooked and may also have low water activity, meaning that the organism is largely inactivated and the resulting product is not favorable for bacterial growth.

The bootstrap technique was used to identify the range of uncertainty associated with sampling error for each risk estimate in each food category; while sensitivity analysis was used to identify those inputs that contributed the most to the quantified uncertainty. Interestingly, the uncertainty analysis revealed that the probability distributions based upon available data were more important sources of uncertainty for our model than were personal assumptions based on expert judgment. However, this might not be the case for other risk assessment models with their specific input assumptions and modeling frameworks. The probability distribution for $\log_{10}$ reduction due to cooking was based upon available data from the USDA-FSIS.[15] Additional data collection can refine the probability distribution considered for this input, and therefore decrease the degree of uncertainty associated with the risk estimate. On the other hand, the model showed little reason to collect additional data on horizontal transmission of SE upon breaking of shell eggs as this input was not selected as one of the key sources of uncertainty.

The initial level of contamination was identified as a key source of uncertainty for extreme values of the estimated risk (e.g., 95th and 99th percentiles of the risk distribution). The input for the initial level of SE contamination in eggs stored at home was based on the results of Latimer *et al.*,[20] who modeled the risk of SE illness associated with various time-temperature scenarios that occur during processing, transportation, and storage of shell eggs. Thus, the home-phase SE model was sensitive to the results from the Latimer model, which in turn was influenced by on-farm and processing phases of the farm-to-fork continuum, in addition to consumer practices. In order to decrease uncertainty in estimated risk from consumption of eggs and egg-containing foods at home, better estimates on the level of SE in contaminated shell eggs in consumers' homes is needed. Unfortunately, such studies are complicated by the low prevalence of SE contamination in shell eggs and limits to microbiological methods, which make enumeration of *Salmonella* difficult at best.

We used Sobol's method as a variance-based technique for identifying key sources of variability. Sobol's method was a valuable technique for sensitivity analysis as applied to our model as it does not require any assumption regarding the functional form of the model. Thus, it can serve as a useful tool for sensitivity analysis of models that are substantially nonlinear, have interactions between inputs, and may have nonmonotonocity in the response. The use of Sobol's method for our case study was particularly appropriate because, typically, the key sources of variability identified for each food category had quite low main effects. Indeed, the sum of main effects for inputs ranged between 0% and 17%. Thus, if a method based on a linear assumption (such as linear regression analysis) was used for sensitivity analysis, we could capture only about 17% of the output variation. The ordering of importance of the inputs based on a sensitivity analysis method such as linear regression analysis is only as good as the associated model coefficient of determination (R^2). In that case, a low value of R^2 between 0 and 0.17 could result in rankings that were not reliable.

Key sources of variability in our model were $\log_{10}$ reduction due to cooking, initial level of contamination, proportion of eggs used as ingredients in each recipe, storage time and temperature at countertop after cooking, and serving size. Among identified key sources of variability, $\log_{10}$ reduction due to cooking, initial level of contamination, and storage time/temperature after cooking were controllable sources of variability. The substantial contribution of $\log_{10}$ reduction due to cooking to probability of SE illness suggested that the degree of undercooking had a great impact on the likelihood of disease resulting from consumption of eggs and egg-containing foods. Time and temperature conditions during preparation were not key inputs affecting the probability of SE illness. Most people refrigerate eggs until use, and the internal temperature of eggs would be relatively low if left out on a countertop for a short period of time before cooking.

The initial level of SE in contaminated shell eggs at home, as a key controllable source of variability, is sensitive to time-temperature abuse during storage and transportation of shell eggs. Latimer *et al.*[20] performed sensitivity analysis on a variety of variable combinations in their on-farm and processing phases of the farm-to-fork continuum risk assessment for SE. For example, sensitivity analysis indicated that the SE contamination level at home was not only associated

with the initial SE contamination level of shell eggs, but also was controlled by temperatures to which the eggs were exposed prior to the home phase. Latimer *et al*.[20] suggested that in order to control the contamination level at home, tighter temperature control and attention to shelf-life issues should be considered. What-if scenario analysis underscored the importance of reduction of contamination by thorough cooking for Category I and II foods such as fried or boiled eggs. Furthermore, risks posed by initial contamination cannot be reduced if foods in Categories I and II are not thoroughly cooked or if raw eggs are used in Category IV foods.

This risk assessment sought to evaluate which of the six types of egg-containing foods present the greatest risk of salmonellosis to consumers and which consumer handling practices contribute the most to the risk of illness associated with SE. The analysis indicates that consumer education should focus on the need to cook egg-containing foods thoroughly in order to reduce risk. In particular, communications should be targeted at the portion of the public that makes a habit of undercooking eggs or using raw eggs in home-made salad dressings and ice cream. Alternatively, public health educators may direct those consumers who prefer partially cooked eggs to use pasteurized shell eggs. The results of the model provide a science-based approach to inform consumer risk communications. However, additional research is needed both to understand why, despite knowledge of the health risks, some consumers persist in consuming undercooked eggs, as well as to identify public health communications that will be effective at protecting this population against *Salmonella* infection.

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